Fatal poisoning involving cyclopropylfentanyl — Investigation of time-dependent postmortem redistribution

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DOI: https://doi.org/10.1016/j.forsciint.2018.11.007

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Time-Dependent Postmortem Redistribution of Cyclopropylfentanyl in Blood and Alternative Matrices

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Formal publication is available in Forensic Science International, vol. 294, pp 80-85; at https://doi.org/10.1016/j.forsciint.2018.11.007

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

The authors express their gratitude to Emma Louise Kessler, MD for her generous legacy that she donated to the Institute of Forensic Medicine at the University of Zurich, Switzerland for research purposes. The authors would like to thank the group from the Department of Forensic Medicine and Imaging, Zurich Institute of Forensic Medicine, for their assistance and the scientists from the Department of Forensic Pharmacology and Toxicology, Zurich Institute of Forensic Medicine, for helpful discussions. This work was supported by the Swiss National Science Foundation (Grant no. 310030_165875).

Abstract

A growing number of fatal overdoses involving opioid drugs, in particular involving fentanyl and its analogues, pose an immense threat to public health. Postmortem casework of forensic toxicologists in such cases is challenging, as data on pharmacodynamic and pharmacokinetic properties as well as reference values for acute toxicities and data on potential postmortem redistribution (PMR) mechanisms often do not exist. A fatal case involving cyclopropylfentanyl was investigated at the Zurich Institute of Forensic Medicine and the Zurich Forensic Science Institute; an unknown powder found at the scene was reliably identified as cyclopropylfentanyl by gas chromatography-infrared spectroscopy (GC-IR). Femoral blood samples were collected at two time points after death; 11 h postmortem (t1) and during the medico-legal autopsy 29 h after death (t2). At the autopsy, additional samples from the heart blood, urine and gastric content were collected. Cyclopropylfentanyl was quantified using a validated liquid chromatography-tandem mass spectrometric (LC-MS/MS) method. Femoral blood concentration of cyclopropylfentanyl at autopsy was 19.8 ng/mL (t1 = 15.7 ng/mL; heart blood concentration at autopsy = 52.4 ng/mL). In the light of the current literature and under the exclusion that no other morphological findings could explain the cause of death, contribution of cyclopropylfentanyl to death was proposed (polydrug use). Significant postmortem concentration increases of cyclopropylfentanyl in femoral blood during 18 h after the first sampling were observed, thus indicating a relevant potential to undergo PMR. A central-to-peripheral blood concentration ratio of 2.6 supports this. Consequently, the current case suggests that postmortem cyclopropylfentanyl concentration should always be interpreted with care.

Keywords

Cyclopropylfentanyl, Fentanyl analogue, Time-dependent postmortem redistribution, LC-MS/MS, Alternative matrices

1. Introduction

The use and misuse of new psychoactive substances (NPS) are a global problem with more than 800 substances having been reported to the United Nations Office on Drugs and Crimes (UNODC) Early Warning Advisory until December 2017 [1]. Synthetic cannabinoids and stimulants make up the largest fraction of these with 68 % reported substances, while synthetic opioids only account for 4 % of those reported in 2017. However, opioid drugs are extensively on the rise over the past years and, albeit small in number, pose an immense threat to public health [2]. This is reflected by a growing number of often fatal overdoses and the awareness that the opioid epidemic was declared a nationwide public-health emergency in the USA [3]. Fentanyl, being about 100 times more potent than morphine, is the strongest opioid licensed for medical use in humans for management of severe pain and during anesthesia [4]. As there has always been concern about its potential for abuse and dependence, fentanyl was placed under international control as a Schedule 1 substance in 1964 under the Single Convention on Narcotic Drugs of 1961 [4]. Shortly after the first synthesis of fentanyl in 1959, an increasing number of analogues have also started to appear. On the one hand during investigations as potential pharmaceuticals and on the other hand emerging on the illicit drug market, mis-sold as illicit heroin or in counterfeit medicines [4]. After a phase in the 1970s and 1980s where fentanyl and its analogues have been the cause for a number of accidental overdoses in the USA, recent years have seen an alarming come-back in both the USA and Europe [5]. Since 2012, 28 new fentanyl analogues have been identified on the European drug market, eight of which were first reported in 2016 and further ten analogues were first identified during 2017 [6]. One of these novel fentanyl analogues is cyclopropylfentanyl; formally reported on the 4th August, 2017 by the European Monitoring Centre for Drugs and Drug Abuse (EMCDDA) for the first time and temporary placed into Schedule 1 of the Controlled Substances Act by the Drug Enforcement Administration (DEA) in January 2018 [7, 8]. It differs from fentanyl by replacement of the propionamide group of fentanyl with a cyclopropanecarboxamide group; chemical structures of fentanyl and cyclopropylfentanyl are displayed in Fig. 1 [9]. Cyclopropylfentanyl has predominantly been detected in powder-form and to a lesser extent in form of liquid solutions (e.g. nasal spray solutions). It has also been identified in tablets, including fake benzodiazepines (e.g. Xanax®) and opioid analgesics (e.g. OxyContin®). The μ-opioid receptor agonist is suspected to show effects in humans, similar to other opioid analgesics such as euphoria, relaxation, analgesia, sedation, bradycardia, hypothermia and respiratory depression [7]. However, the general pharmacology and toxicology of cyclopropylfentanyl remains largely unstudied. This emphasizes the need to publish data on case reports with involvement of cyclopropylfentanyl in order to judge the concentration range of potential acute toxicities. Only very few publications reporting on postmortem or antemortem cyclopropylfentanyl concentrations and analytical challenges are currently available [10-12]. For reliable forensic case interpretation also the concept of postmortem redistribution has to be considered (PMR). This describes the mechanisms and processes that can alter drug concentrations artificially after death, caused by diffusion processes, degradation or drug neo-formation [13]. To the best of our knowledge, there is currently no data on time-dependent postmortem variations available for cyclopropylfentanyl. However, given the structural similarity to fentanyl – a drug that has been shown to undergo extensive PMR – time-dependent concentration changes of cyclopropylfentanyl seem to be likely [14-19]. The aim of the current work was to investigate time-dependent PMR of cyclopropylfentanyl in a single authentic case.

2. Case history

At around 9.00 am the dead body of a 39-year old male was found sitting on a sofa in a crouched position. A previous history of drug abuse was known; presumably regular nasal consumption of cocaine in previous years, but supposedly abstinent around time of death. According to the medical history, the deceased suffered from chronic pain in the region around the cervical spine. As a treatment, he took two tablets of OxyContin® daily (10 mg; one in the morning, one in the evening; active ingredient: oxycodone), ground with a pestle and mortar and nasally consumed. From the coffee table next to the deceased, a small plastic bag with an unknown white power was obtained. The external postmortem examination (at 11.30 am) revealed no injuries or signs of a violent cause of death. Time of death was estimated between 6.5 to 8 h prior to the postmortem medical examination (i.e. between 3.30 and 5.00 am; 4.15 am was set as the reference time of death-point for the purpose of further time interval calculations). Postmortem computed tomography (CT) imaging and autopsy revealed few non-specific signs of intoxication such as weak edema of the brain and blood congested lungs. Additionally, frothy fluid was found in the larynx and respiratory tract.

3. Materials and methods

3.1. Chemicals and reagents

Methanolic solutions of cyclopropylfentanyl (0.1 mg/mL) and the deuterated internal standard (IS) fentanyl-d5 (0.1 mg/mL) were obtained from Chiron AS (Trondheim, Norway) and Lipomed (Arlesheim, Switzerland), respectively. Water was purified with a Purelab Ultra Millipore filtration unit (Labtech, Villmergen, Switzerland), acetonitrile of high-performance liquid chromatography (HPLC) grade was obtained from Fluka (Buchs, Switzerland) and ethyl acetate of pharmaceutical grade from Roth (Karlsruhe, Germany). All other chemicals used, were from Merck (Zug, Switzerland) and of the highest grade available.

3.2. Postmortem samples

Femoral venous blood samples were collected at two time points after death; 11 h postmortem (t1) and during the medico-legal autopsy 29 h after death (t2). At the autopsy, additional samples from the (whole) heart blood, urine and gastric content were collected. All samples were stored at -20 °C until analysis.

3.3. Identification of unknown powder by GC-MS and GC-IR

For gas chromatography coupled to electron ionization mass spectrometry (GC-EI-MS) analysis, the unknown powder sample was basified and dissolved in ethyl acetate (injection of 1 µL of an approximately 1 mg/mL ethyl acetate solution). EI mass spectra (scan range m/z 30–650, after a solvent delay of 2 min) were obtained on an Agilent MSD 5975C (Agilent Technologies, Basel, Switzerland) equipped with an Agilent 7890A GC and an Agilent 7683B

autosampler. Data handling was carried out with the corresponding workstation and Agilent MSD Chem-Station software. The carrier gas was helium at a constant flow rate of 1.3 mL/min. The injector (280 °C) was used in split mode (1:25). Transfer line and ion source were set at 280 °C and 230 °C, respectively. Separations were carried out using a 30 m (length), 0.25 mm (internal diameter), 0.25 µm (film thickness) DB-5MS capillary column. The column temperature was programmed as follows: 80–320 °C with a heat rate of 15 °C/min; then held constant for 4 min at 320 °C; total run time was 20 min. The obtained data were compared to MS-Spectra of the European RESPONSE project as well as with data of the SWGDRUG monograph for cyclopropylfentanyl [20, 21].

For gas chromatography – infrared spectroscopy (GC-IR) analysis, the unknown powder sample was basified and dissolved in ethyl acetate (injection of 1 µL of an approximately 1 mg/mL ethyl acetate solution) subjected to the DiscovIR spectrometer from Spectra Analysis (Marlborough, Massachusetts, USA) coupled with an Agilent 7890A GC and an Agilent 7693 autosampler. The analysis was carried out using the same column type and the same GC conditions as for GC-EI-MS. Transfer line and restrictor were set to 300 °C. The disk was kept at -40 °C, the chamber at $<10^{-4}$ Torr (~ <0.0133 Pa). Solid phase fourier transform – infrared spectroscope (FT-IR) spectra of the isolated cylopropylfentanyl were obtained for direct comparison with attenuated total reflection – infrared spectroscope (ATR-IR) spectra from the National Forensic Laboratory Slovenia from the European RESPONSE project [21].

3.4. Routine systematic toxicological analysis

Routine toxicological analysis was performed on femoral blood and urine collected at t2 (autopsy samples). Initially, urine was screened by a cloned enzyme donor immunoassay (CEDIA®) for common drugs of abuse (opiates, cocaine, cannabis, amphetamines, methadone, barbiturates, benzodiazepines and lysergic acid diethylamide (LSD)), followed by an untargeted liquid chromatography – tandem mass spectrometry (LC-MS/MS) ion trap screening after simple dilution with mobile phase (Bruker amaZon ®; Maurer/Wissenbach/Weber database [22]). Femoral blood was additionally screened for ethanol and other volatile compounds by headspace GC flame ionization detection (HD-GC-FID). Quantification of drugs in femoral blood was performed by LC-MS/MS using a previously validated method [23].

3.5. Sample preparation for PMR investigation

Extractions of samples in triplicate were performed according to Staeheli *et al.* [24]. In short, 20 µL body fluids were used to carry out a two-step liquid-liquid extraction (LLE) with butyl acetate/ethyl acetate (1:1, *v/v*); step 1 at pH 7.4 and step 2 at pH 13.5. After combination of the extracts, all samples were evaporated to dryness and reconstituted in 60 µL mobile phase.

3.6. LC-MS/MS analysis of cyclopropylfentanyl

Quantitative analysis was carried out on a Thermo Fisher Ultimate 3000 UHPLC system (Thermo Fisher, San Jose, CA, USA) coupled to a Sciex 5500 QTrap linear ion trap

quadrupole mass spectrometer (Sciex, Darmstadt, Germany). Instrument settings were adapted from Staeheli *et al.* [24]. Multiple reaction monitoring (MRM) transitions for the ^{12}C isotope of cyclopropylfentanyl were 349 -> 188 (quantifier), 349 -> 105 (qualifier) and 349 -> 77 (qualifier). The quantifier ^{13}C isotope transition was 350 -> 189. Fentanyl-d5 was used as IS. An LC gradient elution was performed using a Phenomenex (Aschaffenburg, Germany) Synergy Polar RP column (length x internal diameter: 100 x 2.0 mm, particle size: 2.5 µm) with 10 mM ammonium formate buffer in water containing 0.1 % (*v/v*) formic acid (pH 3.5, eluent A) and acetonitrile containing 0.1 % (*v/v*) formic acid (eluent B), according to Staeheli *et al.* [24]. The MS was controlled by Analyst® 1.6.3 software (Sciex) and quantitation was performed with MulitQuant® 3.0.2 software (Sciex). Cyclopropylfentanyl concentrations in the gastric content were calculated using the ^{13}C calibration; concentrations of all other samples were calculated with the ^{12}C calibration.

The concentration difference between sampling point t1 and t2 for femoral blood was calculated as percentage difference, defining the mean concentration of triplicate measurements at t1 as 100 %. Statistical significance was tested using the student's t-test (two-tailed distribution, $p < 0.05$).

3.7. LC-MS/MS method validation

The method to quantify cyclopropylfentanyl was validated in peripheral blood in terms of selectivity, calibration model, accuracy, precision, matrix effect, extraction efficiency and limits according to Peters *et al.* as a method for analysis of rare analytes [25].

3.7.1. Selectivity

To check for interfering signals with cyclopropylfentanyl or fentanyl-d5, six blank blood samples from different sources were analyzed. Two blank blood samples spiked with IS (zero samples) were analyzed to check for appropriate IS purity and presence of native analytes.

3.7.2. Calibration model

Calibrators were prepared in duplicates at ten concentration levels; 0.5, 1.0, 5.0, 10, 25, 50, 100, 200, 350 and 500 ng/mL. For calibration levels 1-7 (0.5 – 100 ng/mL) the ^{12}C isotope transition 349 -> 188 of cyclopropylfentanyl was used as quantifier; for calibration levels 6-10 (50 – 500 ng/mL) the ^{13}C isotope transition 350 -> 189 was used as quantifier. The regression lines for both calibrations were based on a linear calibration model with a 1/x weighting to compensate for heteroscedasticity. Back calculation of the mean calibrator concentrations should result in less than ± 20 % deviation to the theoretical concentrations.

3.7.3. Accuracy and precision

Six replicates of quality control (QC) samples at the concentration levels QC low (0.8 ng/mL), QC med (20 ng/mL), QC high (90 ng/mL) and QC high ^{13}C (400 ng/mL) were prepared and

analyzed on one day. Concentrations of QC low and QC med were calculated using the ^{12}C calibration model (0.5 – 100 ng/mL) and concentration of QC high ^{13}C was calculated using the ^{13}C calibration model (50 – 500 ng/mL). QC high concentration was determined with both calibration models. Accuracy was calculated as the percent deviation of the mean calculated concentration at each QC level from the corresponding theoretical value and expressed as bias. A bias within ± 15 % of the nominal values was set as the acceptance criteria. Precision data was calculated as the relative standard deviation (RSD) within the QC levels and would be acceptable if within 15 % RSD.

3.7.4. Matrix effect and extraction efficiency

Matrix effect and extraction efficiency were evaluated at the concentration level of QC med using six blank blood samples from different sources according to the simplified approach described by Matuszewski *et al.* [26].

3.7.5. Limits

The limit of quantification (LOQ) was defined as the concentration of the lowest calibrator (0.5 ng/mL) that had to meet the criteria of signal to noise 10:1 and a bias within ± 20 % of the target value. The limit of detection (LOD) was not systematically investigated.

4. Results and discussion

4.1. Identification of unknown powder by GC-MS and GC-IR

It has recently been highlighted in the literature that it is an analytical challenge to distinguish cyclopropylfentanyl from its structural isomer crotonylfentanyl [11]. Based on identical mass spectral properties, the two isomers cannot be differentiated by MS methods and it is suggested to report the results as cyclopropyl-/crotonylfentanyl in casework [11, 12]. In accordance to this, the current GC-EI-MS analysis of the unknown powder revealed similar library match factors for cyclopropylfentanyl and crotonylfentanyl. However, Mallette *et al.* suggest that differences in the IR-spectra of the two isomers, enables to distinguish cyclopropylfentanyl from crotonylfentanyl [27]. IR spectra of well characterized reference material of both cyclopropylfentanyl and crotonylfentanyl were available from the European RESPONSE project [21]. The data obtained in the current case by FT-IR was consistent with the ATR-IR spectra of cyclopropylfentanyl, thus definite identification of the previously unknown powder could be accomplished. Following this, the current study can reliably report the occurrence of cyclopropylfentanyl, while excluding the occurrence of crotonylfentanyl. Original data are available contacting the corresponding author.

4.2. Routine systematic toxicological analysis

CEDIA® immunoassays in urine were positive for opiates and elevated for LSD (elevated means that the measured value was higher than a negative reference sample, but did not

reach the LSD positive cut-off of 0.5 ng/mL). The LC-ion trap MS screening in urine revealed codeine, diphenhydramine, morphine, noscapine, oxycodone and zolmitriptan. Ethanol content in the femoral blood was found to be 1.27 g/kg (‰), other volatiles were not detected. Quantitative analysis in femoral blood resulted in 480 µg/L codeine, 1000 µg/L diphenhydramine, 14 µg/L morphine, 6.0 µg/L oxycodone and 4.5 µg/L zolmitriptan; 6-monoacetylmorphine, dihydrocodeine, hydrocodone and hydromorphone gave negative results. A positive LSD immunoassay, without indication for LSD consumption in the case circumstances, is often taken as a hint for the intake of fentanyl or fentanyl analogues, based on potential cross-reactivity. In the current case, only an elevated immunoassay result was obtained and although this means the measured value was clearly higher than a negative reference sample, it did not reach the positive cut-off. Hence, this could not be classed as a clear indicator for the intake of fentanyl or a fentanyl analogue. The measured codeine and diphenhydramine concentrations in femoral blood were high, but upon comparison to Launiainen *et al.* they were not necessarily in the lethal range [28]. Within a case cohort of 1903 postmortem cases with codeine detection, fatal concentrations were often considerable higher [28]. Additionally, diphenhydramine concentrations of up to 960 µg/L (within n = 57) were reported with no fatal poisonings within this case cohort [28]. Following this, assuming the intake of fixed dose combination tablets containing codeine, diphenhydramine and noscapine, an overdose of noscapine also seems unlikely. Under the exclusion that no other morphological findings could explain the cause of death, a combined intoxication with ethanol and cyclopropylfentanyl, with potential contribution of codeine and diphenhydramine, was discussed as the most likely cause of death.

4.3. LC-MS/MS method validation

As proposed by Peters *et al.*, a one-day method validation used for a single case was carried out for the quantification of cyclopropylfentanyl [25]. No interfering signals were detected in blank blood and zero samples. For both the ^{12}C and the ^{13}C calibration, the linear regression model gave a R^2 value of > 0.99. Back calculation of the calibrator concentrations resulted in less than ± 15 % deviation to the theoretical concentrations. Other validation results including accuracy, precision, matrix effect and extraction efficiency are given in Table 1. Calibrator 1, defined as the LOQ showed a signal-to-noise ratio of > 10:1 and a bias of 0.5 %. All validation parameters met the previously defined acceptance criteria. It is well known, that validation of a method solely in blood, while quantifying substances in other matrices as well, is generally not sufficient. However, in the current study, cyclopropylfentanyl was included in an existing, fully validated method that covers 83 analytes in 11 postmortem matrices (including the ones tested here) [24]. The former validation has shown, that accuracy and precision data for most analytes were within the specified ranges independent of the matrix, as long as an IS was used. As fentanyl-d5 was employed as IS in the current study, validation in femoral blood was deemed sufficient for quantitative analysis of cyclopropylfentanyl in four different matrices.

4.4. Cyclopropylfentanyl analysis

Femoral blood concentration of cyclopropylfentanyl at autopsy was 19.8 ng/mL (± 0.4 ng/mL across triplicate measurements). Only little reference data is available for interpretation of this

postmortem concentration. Recently, 4 postmortem cases from the UK were reported with a mean cyclopropylfentanyl concentration in femoral blood of 23.7 ng/mL (range 16.6 – 28.9 ng/mL) [11]. Cyclopropylfentanyl was deemed to have contributed to death in all 4 cases, so it seems likely that a similar contribution to death existed in the current case. Lower mean concentrations of cyclopropylfentanyl were reported from the USA; n = 32, mean = 15.2 ng/mL (± 11.9 ng/mL), range 1.4 – 43.3 ng/mL [10]. However analyzed blood was cardiac, iliac, femoral or peripheral blood from one time-point depending on the case availability, and no indication was given whether or not the cyclopropylfentanyl concentration was thought to have contributed to death. Generally it was observed that all submitted postmortem cases were also positive for other drugs, in addition to cyclopropylfentanyl [10]. This holds true for the current case as well, where various other opioids were also detected as detailed above. The advantage of the current work is the availability of additional matrices for analysis; namely samples from the heart blood, urine and gastric content. Distribution of cyclopropylfentanyl across the analyzed matrices is shown in Fig. 2. No postmortem urine concentrations have previously been reported, however recent antemortem data from Canadian substance use disorder clinics are available [29]. Depending on the province, mean urine concentrations of cyclopropylfentanyl of 24 ng/mL (range 1 – 3200 ng/mL), 6 ng/mL (range 3-27 ng/mL) and 16 ng/mL (range 6 – 119 ng/mL) were detected in British Columbia (November 2017), Ontario (November 2017) and Ontario (February to October 2017), respectively [29]. In the current case we found a very low urine concentration of 6.6 ng/mL cyclopropylfentanyl (± 0.3 ng/mL across triplicate measurements). This seems surprising upon comparison to the antemortem data, as we can assume to find a considerable fraction of unchanged cyclopropylfentanyl in urine. A possible explanation would be a relative fast death after intake of the substance, therefore resulting in a short time window for drug distribution and accumulation in urine. A similar reason could also explain the very high quantified concentration in the gastric content (720 ng/mL ± 62 ng/mL across triplicate measurements; semiquantitative as outside the calibration range; total amount of gastric content: approx. 50 mL). As nasal application of cyclopropylfentanyl is assumed, this may have led to some swallowing of the powder or secretion into the acidic gastric content after absorption, leading to such high concentrations [30, 31].

To the best of our knowledge, no time-dependent PMR data for cyclopropylfentanyl is available so far. A frequently used marker for the potential occurrence of PMR is a central-to-peripheral blood concentration ratio (C/P-ratio) of > 1 [32]. Calculating such a ratio with the current concentration data, a C/P-ratio of 2.6 was found (at autopsy: femoral blood concentration = 19.8 ng/mL; heart blood concentration = 52.4 ng/mL). This indicates an extensive PMR-potential of cyclopropylfentanyl. However, a number of recent studies have shown that the C/P-ratio alone might not be a sufficient indicator for postmortem concentration changes as it omits time-dependency and a drugs' physicochemical properties, such as V_d, pKa and protein binding affinity that are thought to highly influence PMR [14, 32-34]. Following this, it is crucial to look at the time-dependent femoral blood concentrations that are available in the current case (Fig. 3). Within 18 h and 10 min – the time difference between the first and second sampling point – a statistically significant 27 % concentration increase of cyclopropylfentanyl in femoral blood was observed (t-test; $p < 0.05$; t1 = 15.7 ng/mL; t2 = 19.8 ng/mL). This supports the previous indication for extensive PMR of cyclopropylfentanyl. A limitation to the applied workflow is the fact that the very early postmortem phase could not be studied due to organizational reasons (i.e. the timeframe between death and admission to the institute). Although it is thought that PMR starts to occur in the first few minutes to hours after

death, the applied method is a valuable tool to assess PMR. A specific redistribution mechanism cannot be proposed based on the current case data. However, butyrfentanyl was previously postulated to be redistributed by passive diffusion [30]. As cyclopropylfentanyl is structurally very similar to butyrfentanyl, a similar redistribution mechanism seems likely and would explain the observed concentration increases in femoral blood over time.

5. Conclusion

A fatal case involving cyclopropylfentanyl revealed significant postmortem concentration increases of cyclopropylfentanyl in femoral blood during 18 h after first sampling. This indicates a relevant potential for PMR, which is supported by a C/P-ratio of 2.6. Consequently, postmortem cyclopropylfentanyl concentration should be interpreted with great caution due to PMR processes. A systematic study covering more cases would be necessary to generate a universal statement on the PMR behavior of cyclopropylfentanyl.

Tables

Table 1: LC-MS/MS method validation results for cyclopropylfentanyl

QC	Concentration [ng/mL]	Calibration	Precision [%]	Bias [%]	Matrix effect ± RSD [%]	Extraction efficiency ± RSD [%]
Low	0.8	^{12}C	5.3	-2.8		
Med	20		2.8	-4.9	110 ± 6.7	90 ± 8.7
High	90		3.9	-5.9		
High	90	^{13}C	3.2	1.5		
High ^{13}C	400		4.3	-6.9		

Figures

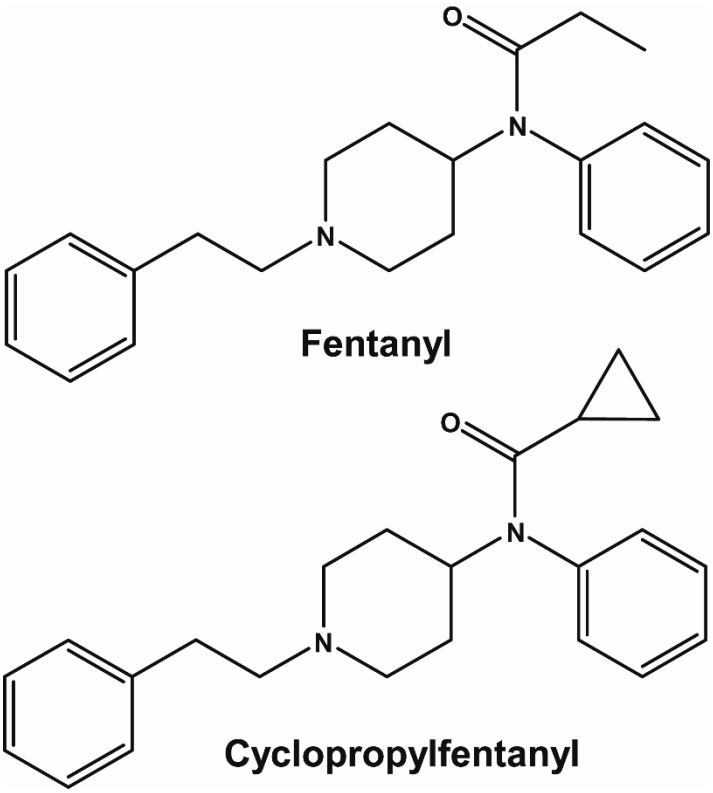

Fig. 1: Chemical structures of fentanyl and cyclopropylfentanyl.

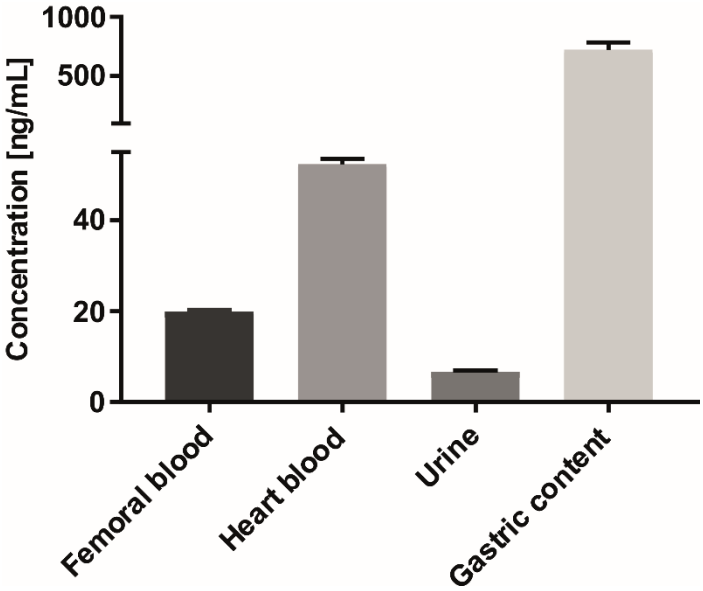

Fig. 2: Distribution of cyclopropylfentanyl across sampled matrices; displayed are the concentration means of triplicate measurements at autopsy (t2).

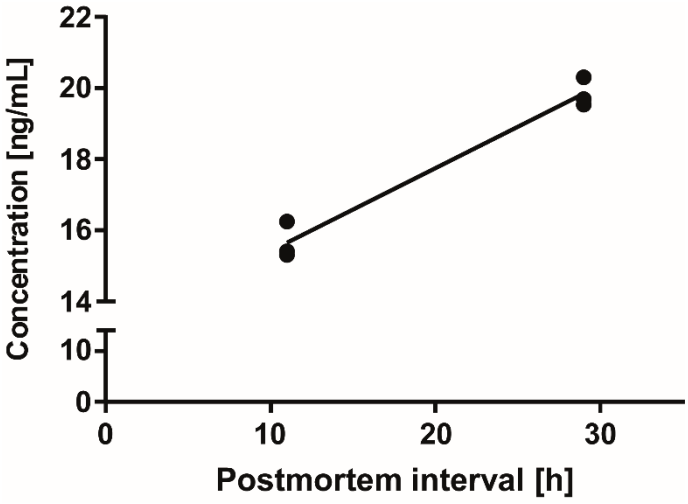

Fig. 3: PMR of cyclopropylfentanyl in femoral blood, displayed as concentration vs. postmortem interval; each dot represents one sample of triplicate measurements; the mean concentrations at each sampling time point were connected with a line.

References

[1] United Nations Office on Drugs and Crime (UNODC), Early Warning Advisory on New Psychoactive Substances. https://www.unodc.org/LSS/Page/NPS, 2018 (accessed 10 August 2018.).

[2] United Nations Office on Drugs and Crime (UNODC), World Drug Report 2018, Vienna, AUT, 2018.

[3] The Lancet, The opioid crisis in the USA: a public health emergency, The Lancet 390(10107) (2017) 2016.

[4] United Nations Office on Drugs and Crime (UNODC), Global SMART Update - Fentanyl and its analogues 50 years on, Vienna, AUT, 2017.

[5] G.L. Henderson, Fentanyl-related deaths: demographics, circumstances, and toxicology of 112 cases, J Forensic Sci 36(2) (1991) 422-33.

[6] European Monitoring Centre for Drugs and Drug Addiction, Fentanils and synthetic cannabinoids: driving greater complexity into the drug situation. An update from the EU Early Warning System, Luxembourg, LUX, 2018.

[7] European Monitoring Centre for Drugs and Drug Addiction, Risk assessment report on a new psychoactive substance: *N*-phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl]cyclopropanecarboxamide (cyclopropylfentanyl), Lisbon, PRT, 2018.

[8] Drug Enforcement Administration, Schedules of Controlled Substances: Temporary Placement of Cyclopropyl Fentanyl in Schedule I. Temporary amendment; temporary scheduling order, Federal register 83(3) (2018) 469-72.

[9] European Monitoring Centre for Drugs and Drug Addiction, EMCDDA-Europol Joint Report on a new psychoactive substance: *N*-phenyl-*N*-[1-(2-phenylethyl)piperidin-4-yl] cyclopropanecarboxamide (cyclopropylfentanyl), Publications Office of the European Union,, Luxembourg, LUX, 2018.

[10] M.F. Fogarty, D.M. Papsun, B.K. Logan, Analysis of Fentanyl and 18 Novel Fentanyl Analogs and Metabolites by LC–MS-MS, and report of Fatalities Associated with Methoxyacetylfentanyl and Cyclopropylfentanyl, J Anal Toxicol (2018) bky035-bky035.

[11] S. Maher, S.P. Elliott, S. George, The analytical challenges of cyclopropylfentanyl and crotonylfentanyl: An approach for toxicological analysis, Drug Test and Anal (2018).

[12] J. Palaty, D. Konforte, T. Karakosta, E. Wong, C. Stefan, Rapid identification of cyclopropyl fentanyl/crotonyl fentanyl in clinical urine specimens: A case study of clinical laboratory collaboration in Canada, Clin Biochem 53 (2018) 164-167.

[13] G. Skopp, Postmortem toxicology, Forensic Sci. Med. Pathol. 6(4) (2010) 314-25.

[14] L. Brockbals, S.N. Staeheli, D. Gascho, L.C. Ebert, T. Kraemer, A.E. Steuer, Time-Dependent Postmortem Redistribution of Opioids in Blood and Alternative Matrices, J Anal Toxicol 42(6) (2018) 365-374.

[15] L. Ceelen, L. De Zwart, M. Voets, V. Hillewaert, J. Monbaliu, G. Teuns, W. Coussement, T. Greway, Postmortem redistribution of fentanyl in the rabbit blood, Am J Forensic Med Pathol 33(2) (2012) 119-23.

[16] C.S. Krinsky, S.L. Lathrop, R. Zumwalt, An examination of the postmortem redistribution of fentanyl and interlaboratory variability, J Forensic Sci 59(5) (2014) 1275-9.

[17] K. Luckenbill, J. Thompson, O. Middleton, J. Kloss, F. Apple, Fentanyl postmortem redistribution: preliminary findings regarding the relationship among femoral blood and liver and heart tissue concentrations, J Anal Toxicol 32(8) (2008) 639-43.

[18] K.N. Olson, K. Luckenbill, J. Thompson, O. Middleton, R. Geiselhart, K.M. Mills, J. Kloss, F.S. Apple, Postmortem redistribution of fentanyl in blood, Am J Clin Pathol 133(3) (2010) 447-53.
[19] V. Palamalai, K.N. Olson, J. Kloss, O. Middleton, K. Mills, A.Q. Strobl, L.C. Thomas, F.S. Apple, Superiority of postmortem liver fentanyl concentrations over peripheral blood influenced by postmortem interval for determination of fentanyl toxicity, Clin Biochem 46(7-8) (2013) 598-602.
[20] Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG), Cyclopropyl Fentanyl, SWGDRUG monographs, 2017.
[21] European Project RESPONSE, Cyclopropyl fentanyl, NPS and related compounds - analytical reports, National Forensic Laboratory, Ljubljana, Slovenia, 2017.
[22] H.H. Maurer, D.K. Wissenbach, A.A. Weber, Maurer/Wissenbach/Weber LC-MSn Library of Drugs, Poisons, and Their Metabolites, Wiley-VCH, Weinheim, GER, 2014.
[23] A.E. Steuer, A.M. Forss, A.M. Dally, T. Kraemer, Method development and validation for simultaneous quantification of 15 drugs of abuse and prescription drugs and 7 of their metabolites in whole blood relevant in the context of driving under the influence of drugs--usefulness of multi-analyte calibration, Forensic Sci Int 244 (2014) 92-101.
[24] S.N. Staeheli, M. Poetzsch, T. Kraemer, A.E. Steuer, Development and validation of a dynamic range-extended LC-MS/MS multi-analyte method for 11 different postmortem matrices for redistribution studies applying solvent calibration and additional 13C isotope monitoring, Anal Bioanal Chem 407 (2015) 8681-8712.
[25] F.T. Peters, O.H. Drummer, F. Musshoff, Validation of new methods, Forensic Sci Int 165(2) (2007) 216-224.
[26] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS, Anal Chem 75(13) (2003) 3019-30.
[27] J.R. Mallette, J.F. Casale, P.A. Hays, Characterization and differentiation of cyclopropylfentanyl from E-crotonylfentanyl, Z-crotonylfentanyl, and 3-butenylfentanyl, Sci Justice (2018).
[28] T. Launiainen, I. Ojanperä, Drug concentrations in post-mortem femoral blood compared with therapeutic concentrations in plasma, Drug Test Anal 6(4) (2014) 308-316.
[29] J. Palaty, D. Konforte, T. Karakosta, E. Wong, C. Stefan, Rapid identification of cyclopropyl fentanyl/crotonyl fentanyl in clinical urine specimens: A case study of clinical laboratory collaboration in Canada, Clin Biochem (2018).
[30] S.N. Staeheli, M.R. Baumgartner, S. Gauthier, D. Gascho, J. Jarmer, T. Kraemer, A.E. Steuer, Time-dependent postmortem redistribution of butyrfentanyl and its metabolites in blood and alternative matrices in a case of butyrfentanyl intoxication, Forensic Sci Int 266 (2016) 170-7.
[31] S.N. Staeheli, M.I. Boxler, A. Oestreich, M. Marti, D. Gascho, S.A. Bolliger, T. Kraemer, A.E. Steuer, Postmortem distribution and redistribution of MDAI and 2-MAPB in blood and alternative matrices, Forensic Sci Int 279(Supplement C) (2017) 83-87.
[32] E. Han, E. Kim, H. Hong, S. Jeong, J. Kim, S. In, H. Chung, S. Lee, Evaluation of postmortem redistribution phenomena for commonly encountered drugs, Forensic Sci. Int. 219(1-3) (2012) 265-71.
[33] M.C. Kennedy, Post-mortem drug concentrations, Intern Med J 40(3) (2010) 183-7.
[34] S.N. Staeheli, D. Gascho, L.C. Ebert, T. Kraemer, A.E. Steuer, Time-dependent postmortem redistribution of morphine and its metabolites in blood and alternative matrices-application of CT-guided biopsy sampling, Int J Legal Med 131(2) (2017) 379-389.